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Regionally different steroid-biosynthesis within materno-fetal circulation units (placentones) of mature human placentas*

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Earlier studies [8] demonstrated the morphological differences between chorionic villi of central and peripheral regions in placentones. Histochemical and biochemical investigations [9] revealed results corresponding to the morphological findings, that is regionally differentiated activity of alkaline phosphatase. They showed that centrally situated villi, which demonstrate a lower degree of maturity than peripherally situated ones, also reacted like less mature villi in respect to activity of alkaline phosphatase.

The purpose of this paper is to examine whether enzymes of steroid-biosynthesis also demonstrate regional differences concerning their own specific activities. This concerns, above all, **aromatising enzyme systems** and **17β-hydroxysteroid-oxidoreductase.** For this reason, we measured the in vitro transformation of Δ-4-androstendione to testosterone and to oestrone/oestradiol-17β by microsomes from tissue of central and peripheral regions of the placentone.

1. Material and Methods

1.1 Tissue

Eight placentas were used from clinically uncomplicated pregnancies, which ended in spontaneous deliveries between the 38th and 40th week of pregnancy. Weight of newborns was normal, APGAR-scores were never under 9.

Curriculum vitae

WOLF DIETRICH LEHMANN, M. D. *born in 1935 in Kiel/ Germany. 1961 Medical Doctor. 1963—1966 clinical resident of the University Hospital for women in Kiel. 1966—1968 Fellowship of the Deutsche Forschungsgemeinschaft at Prof. H. Breuer, Bonn. Experiments about the metabolism of steroids in the liver and placenta. 1969 resident in the University Hospital for women in Ulm (Clinical leaders: Prof.* KNÖRR *and Prof.* LAURITZEN*). Experiments about the conversion of androgens to oestrogens in normal and pathological placentas and the influence of HCG and ACTH on the steroid-biogenesis of the fetal adrenals. Since 1970 Dozent and senior resident in Ulm.*

Membranes and blood were removed immediately post partum and placentas were cut into 2 cm wide sections vertically to the maternal surface.

With the help of a dissecting lens we took biopsies from central and peripheral areas of placentones situated in the middle portion of the placenta. Tissue was then homogenized in 0.25 M sucrose in a glass homogenisator. (The method has been described elsewhere [5].) Remains of connective tissue which could not be homogenized were

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not taken into consideration as regards weight. Microsomal fraction was obtained from the homogenate by ultracentrifugation (Spinco L 50), whereby 1 g (fresh weight) placental tissue was equivalent to 1 ml of the microsomal fraction. Direct weight of the microsomal fraction was determined in the following manner:
After ultracentrifugation, scrupulous decanting of cytoplasma from the residue (microsomal fraction), application of the residue to filter paper, changing the wet filter paper after one minute and weight determination on a microscale.

1.2 Metabolism of (4-^{14}C) Δ_4-Androstendione

In order to study the different enzyme activities of individual segments of placental circulation units, two series of experiments were made. In the **first series** we related the quantity of incubated microsomal fraction (central or peripheral) to the fresh weight of individual placental tissue specimens. In the **second series** of experiments, we determined directly the weight of microsomal fractions gained from placental tissue and used identically weighing portions (5 mg) for incubation. All incubations took place in SOERENSON phosphate buffer (pH 7.35) at 37°C, open to the air in a shaking thermostat. In every case 250 nC (4-^{14}C) Δ_4-Androstendione (specific activity 60 mC/mMol) was dissolved with 0.5 ml microsomal fraction (equivalent to 0.5 g fresh weight placenta) or with 0.5 mg of microsomal fraction and with an NADPH-generating system in 3 ml of 0.15 M SOERENSON phosphate buffer. After 5 minutes incubation, extraction of solutions was performed 3 times with ether/chloroform (3:1). Combined extracts were evaporated in a vacuum.

1.3 Paper chromatography

All experiments were run at 25—27°C. Dry residues stored in methanol were chromatographed on formamid impregnated paper (SCHLEICHER and SCHÜLL 2043 bMg) by Monochlorbenzol. Microchemical reactions: for the identification of oestrone, oestradiol-17β, testosterone and Δ_4-androstendione we performed the following reactions:
1. Oxidation with 3-(or-17β)-hydroxysteroidoxidoreductase (EC 1.1.151) type I from "Sigma Chemicals Co.", St. Louis, USA; steroids were incubated with 2.2 mg of enzyme, 1.8 mg NAD in 1 ml of a 1.0 M carbonate buffer, pH 9.3 at 37°C for 2 hours.
2. Reduction with sodium borate in methanolic solution. (The method has been described elsewhere [5].)
Quantitative analysis: Quantitative analysis of steroids took place on paper chromatograms after measurement of radioactivity with a PACKARD Radiochromatograph scanner, model 7201.

2. Results

As shown in **Tab. I**, (4-^{14}C) Δ_4-androstendione was incubated with microsomal fraction (equivalent to 0.5 g fresh weight) from 12 placentones of 8 placentas. Each incubation took place with a subcellular fraction from the center and then from the periphery of the placentones, so that a direct comparison was possible in each placenta. After a 5 min. incubation period, extraction and paper chromatographical separation, testosterone, oestrone and oestradiol-17β were detected as the main metabolites. 19-hydroxylated metabolites could not be detected on our chromatograms. Activity of 17β-hydroxysteroidoxidoreductase is responsible for the formation of testosterone; transformation of androgens to oestrogens is effected by the aromatising enzyme system. We compared the total obtained oestradiol-17β and oestrone with the substrate (androstendione) and thereby obtained the conversion rate (total oestrogens in percent). **The rate of transformation of androgens to oestrogens** by the microsomal fraction taken from **peripheral tissues** in the placentone amounted on average to **63** $\pm$ **5.4%** and was thus **1.6 times above the value of the central tissue** portions (average 38 $\pm$ 3.4%). The difference is statistically significant ($p < 0.01$, student T-test). The **formation of testosterone** and thus activity of 17β-hydroxysteroidoxidoreductase in the periphery of placental circulation units was likewise approximately **2 times higher than centrally located portions** ($p < 0.01$). In **Tab. II** are shown the results when the rates of transformation were registered, when in each equal weight portions of microsomal fractions from the periphery and center of placentones were incubated. Thereby the **rate of conversion from androgens to oestrogens in the periphery of the placentones was 1.8 times higher than in the center** ($p < 0.01$). As conversion of androstendione to testerone shows, activity of 17β-hydroxy-steroidoxidoreductase lies peripherally 2.3 times higher than centrally ($p < 0.01$).

Tab. I. Conversion of (4-^{14}C) Δ_4-androstendione to testosterone, oestrone and oestradiol-17β by microsomal fractions from tissue specimens obtained peripherally and centrally in the placentone. In each case 250 nC (4-^{14}C) Δ_4-androstendione (specific activity 60 mC/mMol) were incubated with 0.5 ml of a microsomal fraction (equivalent to 1 g fresh weight) from tissue specimens of central and peripheral areas of placentones, from mature human placentas, in the presence of an NADPH-generating system in 2 ml SOERENSON-phosphate-buffer (pH 7.35) open to the air at 37°C for 5 min. Quantitative analysis of metabolites took place, after paper chromatographical separation, by measurement of radioactivity with a PACKARD-radiochromatogram scanner. Results, in each case, are averages from 2 incubations.

Placenta	substrate Δ_4-Androstendione [nC]	remaining Δ_4-Androstendione detected [nC]	Oestrone (Oe_1) produced [nC]	Oestradiol-17β (Oe_2) produced [nC]	testosterone produced [nC]	total amount of Oestrogens produced (Oe_1+Oe_2) [%]
1. peripheral	250	38	50	120	31	68
central		100	20	82	15	41
2. peripheral	250	47	49	116	35	66
central		112	18	72	17	36
3. peripheral	250	52	42	105	33	59
central		118	22	62	16	34
4. peripheral	250	40	39	111	40	60
central		110	23	67	20	36
5. peripheral	250	51	43	101	30	58
central		122	21	64	15	34
6. peripheral	250	40	35	112	34	57
central		66	15	91	18	42
7. peripheral	250	60	52	100	23	61
central		121	19	64	12	33
8. peripheral	250	21	74	114	36	75
central		58	39	71	18	44
9. peripheral	250	42	32	133	28	66
central		81	22	80	19	41
10. peripheral	250	35	60	119	24	71
central		74	22	71	17	37
11. peripheral	250	55	34	122	22	65
central		150	26	68	8	38
12. peripheral	250	61	36	124	20	64
central		103	23	75	10	39
peripheral average value	250	45±11.0	46±14.0	115±12.6	30±6.2	63±5.4
central		101±25.0	22±5.6	66±10.3	15±3.5	38±3.4

Tab. II. Conversion of (4-^{14}C) Δ_4-androstendione to testosterone, oestrone and oestradiol-17β by microsomal fractions from tissue specimens obtained peripherally and centrally in the placentone. In each case 250 nC (4-^{14}C) Δ_4-androstendione (specific activity 60 mC/mMol) were incubated with a 0.5 mg microsomal fraction from tissue specimens of central and peripheral areas of placentones, from mature human placentas, in the presence of an NADPH-generating system in 2 ml SOERENSON-phosphate-buffer (pH 7.35) open to the air at 37°C for 5 min. Quantitative analysis of metabolites took place after paper chromatographical separation by measurement of radioactivity with a PACKARD-radiochromatogram scanner. Results, in each case, are averages from 2 incubations.

Placenta	substrate Δ_4-Androstendione [nC]	remaining Δ_4-Androstendione detected [nC]	Oestrone (Oe_1) produced [nC]	Oestradiol-17β (Oe_2) produced [nC]	testosterone produced [nC]	total amount of Oestrogens produced (Oe_1+Oe_2) [%]
1. peripheral	250	102	43	65	25	43
central		130	27	44	12	28
2. peripheral	250	95	40	71	27	44
central		148	23	43	10	26
3. peripheral	250	90	49	61	29	44
central		150	21	37	14	23
4. peripheral	250	100	39	70	22	43
central		140	22	38	8	24
5. peripheral	250	109	50	79	21	52
central		139	22	41	9	25
Peripheral average value	250	99±6.4	44±4.6	69±6.1	25±3.0	45±3.4
central		141±7.2	23±2.0	41±3.1	11±2.2	25±1.7

3. Discussion

In earlier investigations [4, 5] we demonstrated that at various points during gestation, **placental biosynthesis of oestrogens showed remarkable differences quantitatively.** By incubation of microsomal fraction from 1 g of placental tissue, in vitro conversion rates of androgens (dehydroepiandrosterone and Δ_4-androstendione) to oestrone and oestradiol-17β increase significantly with duration of pregnancy. The increasing protein content, during gestation, in the microsomal fraction indicates that the increase of **enzyme activity is not only a consequence of the increased size of placenta** and syncytial surface, but also that **specific activity of enzyme systems** increases [4]. This finding is confirmed by results from further experiments, which show that by incubating identical quantities of microsomal fraction from placentas at different stages of pregnancy, an increase of the conversion rate with gestation time can be observed [5].

Conversion rates measured from tissue specimens of various areas of the same placenta make it clear that, **in more mature placentas, there are regional differences in enzyme activity.** As follows from Tab. II, there are statistically significant **differences between conversion rates from tissue specimens of central and peripheral regions of the circulation units (placentones).** By use of strictly standarized incubation conditions, double the quantity of substrate (Δ_4-androstendione) was metabolized by microsomal fractions from peripheral portions of placentones than by microsomal fractions from central tissue specimens. As kinetic investigations show [3, 5], Δ_4-androstendione is firstly chiefly reduced by 17β-hydroxysteroidoxidoreductase to testosterone, which is then chiefly transformed to oestradiol-17β by the aromatising enzyme system. A small portion of Δ_4-androstendione is aromatised without a detour through testosterone to oestrone. Results from Tab. I also show accor-

dingly, a distinctly higher yield of oestradiol-17β than from oestrone. C 16-hydroxilated (oestriol) and C 19-hydroxilated metabolites (19-hydroxy-androstendione and 19-hydroxytestosterone) were not found during our incubations. The level of the conversion rates, in correspondence with information from other authors, was clearly dependent on the addition of oxygen and the NADPH-generating system.

Conversion rates which varied regionally correspond well to earlier histological findings [7], which showed a **lower degree of maturity** [1] **in centrally located villi of the placentone.** They show that morphological differences also manifest themselves functionally. From the findings of the greater diameter of the villi and the thereby smaller syncytial surface, it could be assumed that the lower conversion rates of centrally obtained tissue specimens rest purely quantitatively on the fact that in a unit of weigh (1 g) of tissue there is less syncytial and more enzymatical inactive villous stroma. Results of the second series of experiments (Tab. II) in which identical quantities (0.5 mg) of microsomal fractions were incubated, show, however, that the quantitative factor plays a subordinate role. Here also the conversion rate of peripheral tissue is significantly higher. Activities of 17β-hydroxysteroid-oxidoreductase and the aromatising enzyme-system, which vary regionally within the placentone, correspond well to histochemical and biochemical findings about the activity of alkaline phosphatase [9] and glucose-6-phosphate-dehydrogenase (our own, not yet published investigations). These, likewise, showed significantly lower quantities in placentone-centers. These results show that **the peripheral areas of placentones represent the main regions of materno-fetal metabolism,** while their centers are areas of growth and regeneration. The latter is be investigated with audioradiographical studies, which are in progress.

Summary

In eralier investigations, morphological differences were demonstrated between placental villi from central and peripheral regions within materno-fetal circulation units (placentones) of mature human placentas [8]. **Villi in the center of circulation units showed a lesser degree of maturation** [1] **than those in the periphery.**

Histochemical and biochemical studies on activity of alkaline phosphatase [9] demonstrated varied activity, which corresponds to morphological findings; central villi behaved like villi from placentas of pregnancies in the 1st and 2nd trimester.

In this work we therefore tried to determine whether enzymes of steroid-biosynthesis (17β-hydroxysteroid-oxidoreductase and the aromatising enzyme system) **also show regional differences** in respect to their specific activity.

Tissue from central and peripheral portions of placentones, from a total of 8 term placentas, were used for examination after the course of a normal pregnancy and birth. Microsomal fractions prepared from tissue specimens were incubated in two different ways with 4-^{14}C-Δ_4-androstendione.

In the first experiment the microsomal fraction was examined from 0.5 g (fresh weight) centrally and peripherally obtained placental tissue. In the second series of experiments identical weight portions of the microsomal fraction (0.5 mg) were used as the basic material. After incubation, extraction and paper chromatographical separation, we detected quantitatively the main metabolites testosterone, oestrone, oestradiol-17β by measurement of the radioactivity on paper chromatograms. Activity of 17β-hydroxysteroidoxidoreductase is responsible for the formation of testosterone. The conversion of androgens to oestrogens is effected by on aromatising enzyme system.

Results from both series of experiments show consistently a **significantly higher activity** ($p < 0.01$) **of 17β-hydroxysteroidoxidoreductase as well as of the aromatising enzyme system in peripheral tissue specimens.**

Earlier investigations showed that in the course of gestation, placental biosynthesis of oestrogens rises with the increasing maturity of the placenta. Conversion rates measured from tissue specimens of various areas in the same placenta make it clear that **regional differences of enzyme activity are present in the term placenta.** These findings agree with earlier histological and histochemical observations. **Lower enzyme activity is found in the centers of circulation units where morphologi-logically "younger" placental villi were detected.** The results were considered as further **proof that the peripheral areas of placentones represent the main regions of materno-fetal metabolism,** while the centers are predominantly areas of growth and regeneration.

Keywords: Placenta, placental circulation unit (placentone), placental steroid-biosynthesis.

Zusammenfassung

Regional unterschiedliche Steroidbiosynthese innerhalb der materno-fetalen Strömungseinheiten (Placentone) der reifen menschlichen Placenta

In früheren Untersuchungen konnten innerhalb der materno-fetalen Strömungseinheiten (Placentone) reifer menschlicher Placenten **morphologische Unterschiede zwischen den Chorionzotten der zentralen und peripheren Region** nachgewiesen werden [8]. Die Zotten in den Zentren der Strömungseinheiten zeigten einen geringeren Reifegrad [1] als die der Peripheric.

Histochemische und biochemische Studien über die Aktivität der alkalischen Phosphatase [9] ergaben eine den morphologischen Befunden entsprechende unterschiedliche Aktivität: Die zentralen Zotten verhielten sich dabei wie Zotten aus Placenten von Schwangerschaften des 1. und 2. Trimenon.

In der vorliegenden Arbeit sollte geprüft werden, ob auch die Enzyme der Steroidbiosynthese (17ß-Hydroxysteroidoxydoreduktase und aromatisierendes Enzymsystem) **örtliche Unterschiede** hinsichtlich ihrer spezifischen Aktivität **aufweisen.**

Zur Untersuchung gelangte Gewebe aus **zentralen** und **peripheren Anteilen von Placentonen** aus insgesamt 8 Terminplacenten nach unauffälligem Schwangerschafts- und Geburtsverlauf. Die aus den Gewebsproben hergestellte Mikrosomenfraktion wurde in zwei verschiedenen Modifikationen mit 4-^{14}C-Δ_4-Androstendion inkubiert. Im ersten Versuchsansatz kam die Mikrosomenfraktion aus jeweils 0,5 g (Frischgewicht) zentral und peripher entnommenen Placentagewebes zur Untersuchung, in der zweiten Versuchsreihe dienten jeweils gleiche Gewichtsanteile Mikrosomenfraktion (0,5 mg) als Ausgangsmaterial. Nach Inkubation, Extraktion und papierchromatographischer Auftrennung wiesen wir als Hauptmetaboliten Testosteron, Östron und Östradiol-17ß quantitativ durch Messung der Radioaktivität der Papierchromatogramme nach. Die Aktivität der 17ß-Hydroxysteroidoxydoreduktase ist für die Bildung von Testosteron verantwortlich, die Umwandlung von Androgenen zu Östrogenen wird durch das aromatisierende Enzymsystem vollzogen.

Die Ergebnisse beider Versuchsansätze zeigen übereinstimmend eine **signifikant höhere Aktivität** ($p < 0{,}01$) **sowohl der 17ß-Hydroxysteroidoxydoreduktase als auch des aromatisierenden Enzymsystems in den peripheren Gewebsproben.**

In früheren Untersuchungen konnte nachgewiesen werden, daß **im Verlauf der Gestation mit zunehmender Reifung der Placenta die placentare Biosynthese der Östrogene ansteigt.** Die Gewebsproben aus verschiedenen Gebieten ein und derselben Placenta gemessenen Konversionsraten machen deutlich, daß an der Terminplacenta **regionale Unterschiede der Enzymaktivität** vorhanden sind. Diese Befunde stimmen mit früheren histologischen und histochemischen Beobachtungen überein. Die **niedrigere Enzymaktivität findet sich in den Zentren der Strömungseinheiten,** wo auch die **morphologisch „jüngeren" Placentazotten** nachgewiesen wurden.

Die Resultate werden als weiterer Beweis dafür gewertet, daß **die peripheren Bezirke der Strömungseinheiten die Hauptregionen des materno-fetalen Stoffwechsels** darstellen, während die Zentren überwiegend als Orte des Wachstums und der Regeneration anzusehen sind.

Schlüsselworte: Plazenta, materno-fetale Strömungseinheiten (Plazentone), Steroidbiosynthese.

Résumé

Variations régionales dans la biosynthèse stéroïde des unités de circulation foeto-maternelle (placentones) du placenta humain à terme

De précédentes recherches nous ont permis de démontrer dans les unités de circulation foeto-maternelle (placentones) du placenta humain à terme, **l'existence de différences morphologiques entre les villosités choriales des zones centrales et celles des zones périphériques** [8]. Les villosités situées au centre des unités de circulation présentent un degré de maturité moins avancé que celles de la périphérie [1].

Les résultats d'études histochimiques et biochimiques sur l'activité de la phosphatase alcaline [9] sont venus confirmer nos constatations morphologiques. Ils révèlent des différences dans l'activité des deux zones villositaires. Les villosités du centre montrent la même activité que les villosités de placentas du premier et deuxième trimestre de la grossesse.

Le but du présent travail était de rechercher s'il existe également des différences locales dans l'activité spécifique des enzymes de la biosynthèse stéroïde (17ß-hydroxyséroïdoxydoréductase et système enzymatique aromatisant).

Pour notre étude, nous avons choisi 8 placentas provenant de délivrances spontanées, succédant à une grossesse à terme et à un accouchement normal. Nous avons prélevé des fragments de tissus dans les zones centrales et périphériques des placentones. La fraction microsomale obtenue à partir de ces prélèvements fut incubée avec 4-14c-Δ4 androstène-dione de deux façons différentes. Dans une première série d'essais, nous avons examiné la fraction microsomale extraite à partir de fragments de 0,5 mg de tissu placentaire frais, prélevés respectivement dans le centre et la périphérie. Pour la deuxième série, nous avons travaillé chaque fois sur la même quantité (0,5 mg) de fraction microsomale. Après incubation et extraction, nous avons fait une chromatographie de partage sur papier et mesuré la radio-activité du chromatogramme afin de déterminer quantitativement les principaux métabolites, testostérone, oestrone et oestradiol 17ß. La production du testostérone est due à l'activité de la 17ß-hydroxysteroïdoxydoréductase, la conversion des andro-

gènes en oestrogènes est effectuée par le système enzymatique aromatisant.

Les résultats des deux séries d'essais sont concordants et montrent **dans les prélèvements centraux une valeur significativement plus haute** ($p < 0,01$) aussi bien pour l'activité de la 17β-hydroxystéroïdoxydoréductase que pour celle du système enzymatique aromatisant.

Au cours de recherches antérieures, nous avons pu démontrer que **la biosynthèse placentaire des oestrogènes augmente avec l'âge de la grossesse et la maturité du placenta.** Les taux de conversions mesurés sur des prélèvements provenant de différentes zones du même placenta à terme mettent en évidence des **différences locales dans l'activité des enzymes.** Ces résultats concordent avec nos précédentes observations histologiques et histochimiques. **On trouve l'activité enzymatique la plus basse aux centres des unités de circulation où,** comme nous l'avons démontré dans un travail antérieur, **les villosités placentaires sont morphologiquement "plus jeunes".**

Nous considérons ces résultats comme une nouvelle confirmation de notre théorie: **les zones périphériques des unités de circulation forment les principales régions d'échanges foeto-maternels,** tandis que les zones centrales doivent être avant tout considérées comme des lieux de croissance et de régéneration.

Mots clés: Placenta, unités de circulation foeto-maternelle (placentones), biosynthèse stéroïde.

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